about 0.5% to about 1.5% (v/v) of a non-ionic detergent, to produce a solution containing released RNA;

c) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;

d) separating the hybridization complex joined to the solid support from unhybridized sample components; and

e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.

REMARKS

Claims 1-20 are pending; all of the claims stand rejected in a final action mailed August 13, 2001. Claim 19 has been amended. No new matter has been added by these amendments. Applicants have presented arguments describing the cited prior art and the differences between the prior art disclosures and the present invention in response to the rejections of claims 1-18. Entry of this amendment after a final rejection and reconsideration of this application is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 19-20 have been rejected under 35 U.S.C. §103(a), based on the disclosures of Saunders et al., in view of Barany et al. or Reed et al. (U.S. Patent No. 5,908,750). Saunders et al. and Barany et al. had been cited and discussed in earlier office actions, and Reed et al. was cited in the communication mailed August 13, 2001. Reed et al. was further cited as teaching a buffer that includes 0.5 M NaCl (column 16, lines 42-57).

A *prima facie* case of obviousness requires one to (1) determine the content and scope of the prior

art, (2) ascertain the differences between the prior art and the claims at issue and (3) determine the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). The level of ordinary skill in the art of biotechnology or molecular biology is generally considered to be relatively high (*e.g.*, a Ph.D. or equivalent experience). An obviousness determination also requires consideration of whether the prior art (1) would have suggested to those of ordinary skill in the art that they should make the claimed invention, and (2) would have revealed that, in so doing, those of skill in the art would have had a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Claim 19 has been amended as supported in the disclosure at page 24, line 2 to page 25, line 27. The specific changes include: as the transition phrase, "consisting essentially of" in place of "comprising" and in the mixing step b) adding the phrase "to produce a solution containing released RNA". Also, to further clarify the method steps, step b) has been divided into steps b) and new step c), with the subsequent steps re-lettered as steps d) and e). Amended claim 19, step c) is another mixing step in which the solution containing released RNA is mixed with a solid support to which is joined an immobilized oligonucleotide for forming a stable immobilized oligonucleotide:RNA hybridization complex. That is, amended claim 19, step b) is a first mixing step that uses soluble components (a buffer at a pH of about 6.5 to about 8.5, about 150 mM to about 1M of a soluble salt, and about 0.5% to about 1.5% (v/v) of a non-ionic detergent) to produce a solution containing released RNA; and step c) mixes the solution containing released RNA with a solid support having an attached oligonucleotide to form a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions.

The Examiner stated that the "comprising" term of claims 19 and 20 makes the claims broad enough to encompass the methods of Saunders et al. and Barany et al. The change of the transition phrase to "consisting essentially of" should overcome this objection.

The Examiner further stated that "a review of the art at the time the invention was made reveals a plethora of references teaching the claimed buffer concentrations" although only three references have been cited. With regard to claim 19, the Examiner's remarks appear to be directed only to the soluble buffer components in step b), independent of the other steps of the claimed method. None of the cited art

includes a step of mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide to form a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions.

The Saunders et al. and Barany et al. have been discussed in greater detail in earlier responses. To avoid repeating these descriptions, Applicants respectfully refer the Examiner to the Amendments filed May 23, 2001 and December 12, 2000. Here, the teachings of Saunders et al., Barany et al., and Reed et al., are summarized and compared to the claimed method.

Saunders et al. teach a method of isolating RNA that uses a buffer solution containing 75 mM NaCl, 25 mM Na_2EDTA and an ionic detergent (0.1% Sarkosyl NL-97) (column 5, lines 34-37). Saunders et al. teach a method that extracts leukocyte pellets with a buffer combined with phenol, followed by proteinase K digestion, and then additional extractions using phenol, and a mixture of chloroform and isoamyl alcohol (column 5, lines 34-47). The extracted nucleic acids are precipitated from solution, dissolved and then poly(A+) RNA is selected using oligo(dT) chromatography (column 5, line 48 to column 6, line 16). Unlike the claimed method, Saunders et al. do not teach (1) use of a soluble salt at 150 mM to 1M concentrations, or (2) use of non-ionic detergent at about 0.5% to about 1.5% (v/v), or (3) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide to form a stable immobilized oligonucleotide:RNA hybridization complex.

Barany et al. teach a method of isolating DNA from cell nuclei that uses a buffer containing 2 mM EDTA and 0.45% each of non-ionic detergents (NP40 & Tween 20) (see column 42, lines 20-36). Barany et al. teach a method that includes enzymatic digestions of protein and RNA followed by "sequential extractions with phenol, phenol/chloroform, chloroform, [and] n-butanol" and precipitation of the isolated genomic DNA (see column 42, lines 40-45). Unlike the claimed method, Barany et al. do not teach (1) use of a soluble salt at 150 mM to 1M concentrations, or (2) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide to form a stable immobilized oligonucleotide:RNA hybridization complex. Indeed, the method of Barany et al. removes or destroys RNA from the genomic DNA that is isolated.

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Reed et al. teach methods to release cellular proteins for further analysis (column 13, lines 18-39, and column 16, line 44 to column 17, line 13). These methods use a buffer containing NaCl (0.15 M or 0.5M) and Trition X-100 (1% or 0.1%) and other components (EDTA, and protease inhibitors such as pepstatin, PMSF, aprotinin, and leupeptin). Following lysis, the cellular proteins were further analyzed by determining protein concentration (column 13, lines 31-33), electrophoretic separation by size and transfer to filters (column 13, lines 34-39), and characterization by antibody-binding reactions (column 13, lines 39-67). Alternatively, following lysis, the cellular proteins were further analyzed by binding reactions that measure the ability of proteins to bind to labeled DNA fragments causing a shift in the mobility of the DNA fragments when analyzed by electrophoretic size separation (column 16, line 58 to column 17, line 13). Unlike the claimed method, Reed et al. do not teach a method of preparing RNA, but instead teach methods for preparing proteins. Reed et al. do not teach or suggest mixing a solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide to form a stable immobilized oligonucleotide:RNA hybridization complex.

As demonstrated by the above discussion of the Saunders et al., Barany et al. and Reed et al. disclosures, the content and scope of the cited art differs from the claimed methods in significant ways. Although the Examiner stated at page 3, second paragraph, of the Office Action that "All of the cited reference provide the motivation for extraction of RNA from cells", Applicants respectfully disagree. Barany et al.'s method *destroys* RNA while isolating genomic DNA and Reed et al.'s methods isolate *proteins*. Therefore there appears to be no motivation to combine the disclosures of these references with the primary reference. Based on the differences between the prior art disclosures and the claimed method, and the lack of motivation to combine them, it appears that the Examiner has impermissibly used hindsight based on the Applicants' disclosure to construct one portion of the claimed method (a buffer solution). Therefore, the Examiner has not provided a *prima facie* case of obviousness in rejecting claims 19 and 20, and Applicants respectfully request that the rejections be withdrawn.

In addition, the Reed et al. reference was cited for the first time in the final office action mailed

August 13, 2001, "to further teach that the claimed buffer and salt concentrations were known in the art for extraction methods such as the ones claimed...." (Office Action, page 3, first full paragraph). This citation was not necessitated by the Applicants' amendment of the claims (MPEP 706.07(a)) because the buffer and salt concentrations were in the claims submitted in December 2000 with the RCE application. Because Reed et al. in fact disclose *protein* extraction methods, Applicants respectfully request that finality be withdrawn to prevent a "hasty and ill-considered final rejection" (MPEP 706.07).

Claims 1-18 stand rejected under 35 U.S.C. §103(a), based on the combined disclosures of Eskola et al. (Clin. Biochem., 1994, 27:373-379), Kacian et al. (US Pat. 5,399,491) and Saunders et al. in view of Rowley et al. (US Pat. 5,487,970), Morris et al. (US Pat. 5,529,925), von Lindern et al. (Molec. Cell. Biol., 1992, 12:3346-3355), Goddard et al. (Science, 29 Nov 1991, pp. 1371-1374), Ohki et al. (US Pat. 5,580,727) and either Barany et al. or Reed et al.

To establish a *prima facie* case of obviousness, one must (1) determine the content and scope of the prior art, (2) ascertain the differences between the prior art and the claims at issue and (3) determine the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). In biotechnology/molecular biology inventions, the level of ordinary skill in the art is generally relatively high as discussed above. In determining obviousness, one must also consider whether the prior art would have suggested to one skilled in the art to make the claimed invention, and would have revealed a reasonable expectation of success in making the claimed invention. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

With regard to claims 1-18, the Examiner has cited as the primary references the combination of Eskola et al., Kacian et al., and Saunders et al. to be considered in view of Rowley et al., Morris et al., von Lindern et al., Goddard et al., Ohki et al., and either Barany et al. or Reed et al.

(1) The contents and scope of the prior art references are summarized as follows.

Eskola et al. teach a method of detecting translocations between the human *abl* gene of chromosome 9 and the bcr gene of chromosome 22 by using the reverse transcriptase-polymerase chain

reaction (RT-PCR) to amplify portions of the translocation sequence, followed by detection of the amplified nucleic acid using a method that *requires* at least two probes for detection. One probe binds 5' of the translocation splice junction and one probe binds 3' of a splice junction (Abstract, lines 13-16; page 374, col. 1, lines 14-16 and p. 375, col. 2, lines 26-31). Eskola et al.'s method requires one probe to bind the sequence to be detected to a solid surface, and one labeled probe that binds to the bound complex (page 375, col. 2, lines 23-48). Both probes are required to bind for the amplified sequence to be detected: if one one of the two probes binds, the sequence cannot be detected. That is, if only the probe that binds the sequence to the solid surface binds, then the amplified sequence will not be labeled and cannot be detected. Similarly, if only the labeled probe binds the sequence, then the amplified sequence will be labeled but will be indistinguishable from the mixture that includes unbound labeled probe and any other nucleic acids in the mixture (e.g., translocated and untranslocated sequences).

Kacian et al. disclose methods of amplifying nucleic acids by synthesizing multiple copies of a target nucleic acid sequence under conditions of substantially constant temperature to produce multiple RNA copies of the target sequence that autocatalytically generate additional copies. In the Background section (column 1, lines 33-40), Kacian et al. introduce their invention by describing the importance of techniques for detection and quantitation of nucleic acid sequences for a variety of practical applications, including detecting genetic abnormalities.

Saunders et al. is summarized above with regard to the rejections of claims 19 and 20. The Examiner is respectfully referred to that summary to avoid repeating it here.

Rowley et al. teach methods of detecting genetic rearrangements that rely on detection of a gene fragment or message of aberrant size or pattern (Abstract, lines 10-17; col. 3, lines 55-60; col. 4, lines 1-7 and 32-42; col. 5, lines 4-8; col. 21, lines 43-46). Rowley et al. use chromatographic separation, filter binding and probe binding methods (e.g., Southern and Northern blotting) to detect the aberrant nucleic acids by using a "beakpoint-spanning" probe (col. 3, lines 17-19 and 31-33) to differentiate between normal and leukemic cells.

Morris et al. teach detection of a gene fusion sequence by using nucleic acid amplification followed

by detection using two probes: one probe binds to one of the gene fusion partners (NPM sequence) and the other probe binds to the other gene fusion partner (ALK sequence). Both probes must bind to indicate the presence of the NPM/ALK fusion (col. 2, lines 1-30, particularly lines 28-30). In another teaching, Morris et al., use a single probe that spans the NPM/ALK fusion junction to detect the fusion (col. 2, lines 31-33; col. 15, lines 65-67). This alternative method is similar to Rowley et al.'s "breakpoint-spanning" probe for detection of the abnormal junction.

von Lindern et al., Goddard et al., and Ohki et al. each teach various genetic translocations. Discovery or characterization of a genetic translocation is not claimed.

von Lindern et al. describe isolation and characterization of a gene (*set*) that was fused in leukemic cells to another gene (*can*) which resulted in a chimeric *set-can* transcript (page 3346, col. 2, last sentence before "MATERIALS AND METHODS").

Ohki et al. teach amplification and detection of a cDNA containing a t(8;21) fusion site by using a probe that contains the fusion site (col. 3, line 55 to col. 4, line 7), which is similar to the single-probe detection systems described by Rowley et al. and Morris et al. Ohki et al. also describe amplifying a sequence containing the fusion site and then detecting the amplified DNA by gel electrophoretic separation of the DNA by size and ethidium bromide staining of the DNA (col. 4, lines 19-26).

Goddard et al. describe cDNA cloning and analysis of clones containing t(15;17) translocation sequences (p. 1371, col. 1, first paragraph and col. 3, first full paragraph).

Barany et al. describe a method of isolating genomic DNA which is summarized above with regard to the rejections of claims 19 and 20. The Examiner is respectfully requested to refer to that discussion to avoid repeating it here.

Reed et al. describe methods of extracting cellular proteins for further characterization of the proteins, which are summarized above with regard to the rejections of claims 19 and 20. The Examiner is respectfully requested to refer to that discussion to avoid repeating it here.

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(2) The differences between the prior art and the claims at issue are summarized below.

Claim 1 is for a method for detecting a fusion nucleic acid consisting essentially of the steps of:

a) providing a sample containing a first single-stranded fusion nucleic acid comprising a splice junction;

b) contacting under nucleic acid amplification conditions: the first single-stranded fusion nucleic acid, a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and at least one nucleic acid polymerase activity;

c) amplifying the fusion nucleic acid in an isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises: a complementary splice junction site, a first probe binding site located 3' to and not overlapping the complementary splice junction site, and a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

Claims 2-8 depend from claim 1.

Claim 9 is for a method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation. This method consists essentially of steps that are similar to those of claim 1, but substitute "fusion mRNA transcript" for "fusion nucleic acid." For example, the method provides a sample that contains a fusion mRNA transcript (step a); the contacting step (step b) contacts the fusion mRNA transcript and a first primer which hybridizes to the fusion mRNA transcript; the amplifying step

(step c) amplifies the fusion mRNA transcript to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site; and that the detecting step (e) detects a hybridization complex that indicates the presence of the fusion transcript in the sample.

Claims 10-18 depend from claim 9, directly or through claims that ultimately depend from claim 9.

The claims must be considered as a whole, i.e., for all of the elements they include, when comparing the teachings of the prior art to the claimed methods. The differences between the prior art and the pending method claims discussed below concentrate mainly on independent claims 1 and 9. If a prima facie case of obviousness is not established for the independent claims, then the dependent claims cannot be found obvious.

The method taught by Eskola et al. requires two probes that bind to two sites of the nucleic acid containing a splice junction site for that nucleic acid to be detected. In contrast, Applicants' claimed methods (see claims 1 and 9, steps c and d) specify that the nucleic acid to be detected includes a complementary splice junction, a first probe binding site (3' of the splice junction site), and a second probe binding site (5' of the splice junction site) and that the probe binds to *either* the first probe binding site *or* the second probe binding site. The "either ... or" language in claims 1 and 9, steps d) make clear that the probe binds to only one of the probe binding sites, unlike the two-probe binding system of Eskola et al. The disclosure of Eskola et al. not only does not suggest the claimed methods but teaches away from the single probe detection steps because if only one probe of Eskola et al.'s two-probe system were used, the nucleic acid would not be detectable. Also, based on the teachings of Eskola et al., one skilled in the art at the time the invention was made would not have predicted success for Applicants' claimed methods because detection as taught by Eskola et al. required *two* probes binding to *two* sites for successful detection of the translocation nucleic acid sequence. Therefore, the Eskola et al. reference does not suggest the present method claims.

Kacian et al. do not suggest the present invention because the disclosure of Kacian et al. provides no details that would have suggested the claimed methods to one skilled in the art at the time of the invention. Kacian et al.'s statement (column 1, lines 33-40) that detection and quantitation of nucleic acid sequences is an increasingly important technique, followed by the disclosure of amplification methods do not suggest the presently claimed methods which specify structural elements used to detect the presence of a fusion nucleic acid or RNA in a sample. Kacian et al. included an example (Example 15, column 41, line 34 to column 42, line 2) that compares amplification methods practiced using two different reverse transcriptases (derived from AMV and MMLV) and using primers for amplification of a human BCL-2 chromosomal translocation. Although this example describes amplification of some portion of a translocation sequence, it does not suggest the present method that specifies amplifying the fusion nucleic acid or fusion transcript to produce second nucleic acid strands that include a complementary splice junction site, a first probe binding site located 3' to and not overlapping the complementary splice junction site, and a second probe binding site located 5' to and not overlapping the complementary splice junction site. No such details are suggested by Kacian et al.'s example. Furthermore, Kacian et al.'s example does not suggest in the hybridizing steps of the present claims (steps d in claims 1 and 9) in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid which is detected to indicate the presence of the fusion nucleic acid in the sample. Instead Kacian et al.'s example presents detection results to show the relative efficiencies of amplification using either the AMV reverse transcriptase or the MMLV reverse transcriptase (column 41, Table 22). The present claimed methods include amplifying steps (steps c of claims 1 and 9) that specify that an "isothermal nucleic acid amplification reaction" is used, but the disclosure of such an amplification reaction does not make the present method claims obvious, even if combined with the disclosures of the other cited art. Nor would Kacian et al.'s disclosure have revealed a reasonable expectation of success in making the claimed invention to one skilled in the art at the time Applicants' invention was made, even if it were combined with the other prior art because the combined references do not suggest the structural elements of the present claims 1 and 9 in steps c and d.

Saunders et al. disclosure of a method to isolate RNA, which even if combined with the disclosures of Eskola et al. and Kacian et al. would not suggest the present methods. Saunders et al. provide no disclosure that would fill in the gaps in the disclosures of Eskola et al. and Kacian et al. relative to the claimed methods of claims 1 and 9 described above.

Rowley et al.'s disclosed method requires a "breakpoint-spanning" probe that binds to a splice junction sequence. In contrast, Applicants' claimed methods do not use a probe that hybridizes across the splice junction but instead use a probe that binds to *either* the first probe binding site (3' of the splice junction) *or* the second probe binding site (5' of the splice junction) as described above relative to claims 1 and 9, steps d. The disclosure of Rowley et al. not only does not suggest the claimed methods but teaches away from using a probe that binds either 3' of or 5' of the splice junction site because Rowley et al.'s method teaches detection by using a probe that hybridizes to the splice junction site.

Morris et al.'s two methods are unlike Applicants' claimed methods, particularly steps d of claims 1 and 9. In one version, Morris et al. teach a two-probe system that is similar to the two-probe system of Eskola et al. because *both* probes are required to bind to detect the splice junction-containing nucleic acid. In the other version, Morris et al. teach a single-probe system, similar to Rowley et al., because the probe hybridizes to the splice junction site sequence. This is unlike Applicants' methods that hybridize a single probe to the first probe binding site or the second probe binding site, neither of which overlaps the splice junction sequence.

von Lindern et al. describe a specific translocation that results in a chimeric transcript, but provide no disclosure that fills in the gaps left by the disclosures of Eskola et al., Kacian et al. and Saunders et al. with regard to the present claimed methods as discussed above.

Goddard et al.'s disclosure of cDNA cloning and analysis of clones containing specific translocation sequences provides no additional disclosure that makes the present method claims obvious. At best, Goddard et al.'s disclosure might provide an invitation to experiment to detect the sequences they disclose.

Ohki et al. teach amplification of a nucleic acid containing a specific fusion site and detection by

using a probe that binds to the splice junction site. Like the methods of Rowley et al. and Morris et al., Ohki et al. do not suggest the present methods that rely on binding a probe to either a binding site located 3' of or 5' of the splice junction site. Like the disclosures of Rowley et al. and Morris et al., the probe system of Ohki et al. teaches away from the present method claims (specifically steps d of claims 1 and 9).

Barany et al. describe a method of isolating genomic DNA that destroys RNA in the process. Therefore, Barany et al. must teach away from the methods of claims 9-18, which are drawn to methods of detecting a fusion mRNA. Barany et al. provide no information in their methods of DNA isolation that would make method claims 1-8 obvious because Barany et al. provide nothing that makes the independent claim obvious even if the Barany et al. disclosure is combined with the other cited art. Claim 6, to which the Barany et al. disclosure is most relevant, depends from claim 1.

Reed et al. disclose method of lysing cells to release proteins for further analysis. Reed et al. provide no information that would suggest the present method claims 1 and 9, even if combined with the primary cited prior art because Reed et al.'s protein detection methods provide no information to fill in the gaps in that art relative to the presently claimed methods.

Based on the above discussion of the scope and content of the cited prior art, and the ascertained differences between the prior art and the elements of the present method claims, Applicants respectfully submit that the present claimed methods would not have been obvious at the time the invention was made to one skilled in the art of molecular biology. Indeed, many of the cited references teach away from the claimed methods, particularly steps d in claims 1 and 9. Furthermore, one skilled in the art, based on the disclosures in the cited prior art would not have had an expectation of success using the present claimed methods because many of the cited references would have predicted that detection relying a hybridization step as defined in steps d in claims 1 and 9 would not have been successful because detection in the prior art either required two probes binding to two portions of the spliced nucleic acid sequence or a single probe binding to or spanning the splice junction site. Therefore, the Examiner has failed to establish a *prima facie* case of obviousness for claims 1-18 even when nine or ten references were combined. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. 103(a).

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Claims 1 and 9 use the transition phrase "consisting essentially of" which is used to mean that additional method steps that do not materially change the basic and novel characteristics of the present invention may be included in the methods of the invention. Based on the above discussion that shows the differences between the cited prior art disclosures and the claimed methods, Applicants respectfully submit that the claimed methods are not made obvious by the cited prior art. Therefore, focusing the discussion on whether a phenol/chloroform extraction step would or would not materially change the basic and novel characteristics of the presently claimed methods seems unnecessary.

CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims are in condition for allowance. Accordingly, allowance of the application is earnestly solicited. The undersigned has made a good-faith effort to address all the points raised in this Office Action and to place the claims in condition for allowance. If minor matters remain that could be resolved by telephone interview, the Examiner is invited to contact the undersigned at the number below.

Applicants believes there is no fee due in connection with the filing of this Amendment. However, if Applicants are in error and a fee is required, please debit Deposit Account No. 07-0835 the appropriate amount.

Respectfully submitted,

Date: November 13, 2001

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AMENDMENT AFTER FINAL
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Marked Up Copy of Claims
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Marked Up Copy of Amendments

IN THE CLAIMS:

Kindly amend **Claim 19** as shown in the following marked-up claims.

1. (Reiterated) A method for detecting a fusion nucleic acid consisting essentially of the steps of:

a) providing a sample containing a first single-stranded fusion nucleic acid comprising a splice junction;

b) contacting under nucleic acid amplification conditions:

the first single-stranded fusion nucleic acid,

a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and

at least one nucleic acid polymerase activity;

c) amplifying the fusion nucleic acid in an isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to

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Christine A. Gritzmacher

sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

2. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

3. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, wherein the amplifying step includes contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a second primer binding site located 3' to both the complementary splice junction and the first probe binding site, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (Reiterated) The method of Claim 1, wherein the oligonucleotide probe binds to the second probe binding site and does not form a stable hybridization complex with the first single-stranded fusion nucleic acid.

5. (Reiterated) The method of Claim 1, wherein the fusion nucleic acid is a *bcr-abl* fusion mRNA and wherein the oligonucleotide probe binds to a *bcr*-derived nucleotide base sequence in the second nucleic acid strands.

6. (Reiterated) The method of Claim 1, wherein step a) includes preparing RNA from the sample

containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or chloroform.

7. (Reiterated) The method of Claim 6, wherein the fusion nucleic acid is mRNA.

8. (Reiterated) The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.

9. (Reiterated) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation consisting essentially of the steps of:

a) providing a sample containing a fusion mRNA transcript comprising a splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site, and

at least one enzyme having nucleic acid polymerase activity;

c) amplifying the fusion mRNA transcript in a nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, wherein the first probe binding site is derived from a second chromosomal region, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site is derived from a third chromosomal region and overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion transcript, thereby forming a hybridization complex of the probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. (Reiterated) The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. (Reiterated) The method of Claim 9, wherein the first probe binding site and the second probe binding site are derived from different locations on the same chromosome in a eukaryotic cell, and the fusion mRNA transcript detected results from an intrachromosomal translocation.

12. (Reiterated) The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript detected results from a translocation involving different chromosomes.

13. (Reiterated) The method of Claim 12, wherein the fusion mRNA transcript results from a translocation of human chromosomes selected from the group consisting of: t(1;19), t(2;5), t(2;13), t(4;11), t(6;9), t(8;21), t(9;11), t(9;22), t(11;14), t(11;19), t(11;22), t(12;21), t(14;18) and t(15;17) translocations.

14. (Reiterated) The method of Claim 13, wherein the fusion mRNA transcript results from a human t(9;22) translocation and the oligonucleotide probe comprises a *bcr*-derived sequence or an *abl*-derived sequence.

15. (Reiterated) One or more oligonucleotides suitable for use in the method of Claim 14, have a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

16. (Reiterated) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. (Reiterated) The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

18. (Reiterated) The method of Claim 9, wherein the amplifying step also amplifies an internal control transcript in the sample by using the first primer and then hybridizing a second oligonucleotide probe which

hybridizes to the complement of the internal control transcript but does not hybridize to the complement of the fusion mRNA transcript thereby forming in internal control hybridization complex, and wherein the detecting step also detects the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Amended 4 times) A method of preparing a sample containing RNA suitable for amplification, [comprising] consisting essentially of the steps of:

a) providing a biological sample comprising unpurified RNA;

b) mixing the biological sample with a solution consisting essentially of:

a buffer at a pH of about 6.5 to about 8.5,

about 150 mM to about 1M of a soluble salt, and

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, [and] to produce a solution containing released RNA;

c) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;

[c)] d) separating the hybridization complex joined to the solid support from unhybridized sample components; and

[d)] e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. (Reiterated) The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.